

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12P 21/02, C12S 3/16	A1	(11) International Publication Number: WO 90/08195 (43) International Publication Date: 26 July 1990 (26.07.90)
(21) International Application Number: PCT/US90/00283 (22) International Filing Date: 11 January 1990 (11.01.90) (30) Priority data: 297,100 13 January 1989 (13.01.89) US (71) Applicant: PRESIDENT AND FELLOWS OF HARVARD COLLEGE [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US). (72) Inventors: OLSEN, Bjorn, R. ; 48 Vose Hill Road, Milton, MA 02186 (US). NINOMIYA, Yoshifumi ; 76 Grosvenor Road, Needham, MA 02192 (US). KIMURA, Tom-aotsu ; 21-5 Higashi Ashiya-cho, Ashiya-shi, Hyogo-ken 659 (JP).		(74) Agent: FURLONG, Robert, W.; Fish & Richardson, One Financial Center, Suite 2500, Boston, MA 02111-2658 (US). (81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i>
(54) Title: MONOCLONAL ANTIBODY TO HUMAN TYPE IX COLLAGEN (57) Abstract Oligopeptides corresponding to segments of human Type IX collagen are useful as antigens in producing monoclonal antibodies which bind immunologically to human Type IX collagen and fragments derived from it and which can be used to detect their presence in biological fluids such as synovial fluid and serum.		

MONOCLONAL ANTIBODY TO HUMAN TYPE IX COLLAGEN

This invention relates to purified oligopeptides corresponding to fragments of human Type IX collagen and to monoclonal antibodies which bind specifically to human Type IX collagen.

Type IX collagen is one of a distinct class of extracellular matrix proteins associated with the surface of collagen fibrils in cartilage. In patients suffering from degenerative cartilage diseases such as rheumatoid arthritis and osteoarthritis, the Type IX collagen is disrupted, so that it or fragments of it are no longer in their normal location and can instead be found in the intra-joint fluid, for example. This abnormal condition of Type IX collagen is symptomatic of the disease.

A number of earlier publications describe the structure and composition of chicken Type IX collagen, such as Konomi, et al., J. Biol. Chemistry Vol. 261, 6742-6746 (1986); McCormick, et al., Proc. Natl. Acad. Sci. USA Vol. 84, 4044-4048 (1987); Vasios, et al., J. Biol. Chemistry Vol. 263, 2324-2329 (1988); Svoboda, et al., Proc. Natl. Acad. Sci. USA Vol. 85, 7496-7500 (1988); Olsen, et al., Anal. of NY Acad. Sci., Vol 460, pp 141-153 (1985); Ninomiya, et al., Proc. Natl. Acad. Sci. USA, Vol. 81, pp 3014-3018, (1984); Lozano, et al., Proc. Natl. Acad. Sci. USA Vol. 82, pp.4050-4054 (1985); Ninomiya, et al., Biochemistry, Vol. 24, pp. 4223-4229, (1985); van der Rest, et al., J. Biol. Chemistry, Vol 260, pp 220-225 (1985); Huber, et al., J. Biol.

-3-

detecting the presence or absence of Type IX collagen, $\alpha 1$ (IX) collagen chains or fragments thereof, thus serving as a diagnostic tool for the presence or absence of a degenerative cartilage disease. In addition, the antibodies can be used to screen drugs for use against such disease.

Figs 1A and 1B show the amino acid sequence of the human $\alpha 1$ (IX) collagen chain as deduced from the cloned cDNAs.

The cDNAs coding for the protein sequence of Fig. 1 were obtained by conventional extraction and isolation of RNA from chondrocytes derived from a costal cartilage specimen obtained from a female patient, followed by synthesis and cloning of the cDNA according to procedures well known in the art. The human cDNA library was screened by filter hybridization, positive phages purified, and recombinant DNA isolated using standard procedures, and nucleotide sequence analysis was performed by the Maxam and Gilbert procedure as well as by the dideoxy chain termination technique.

From the sequence of Fig. 1 the following segments were selected as containing the most promising epitopes:

Example 1

Gly-Arg-Ala-Pro-Thr-Asp-Gln-
His-Ile-Lys-Gln-Val-

Example 2

Glu-His-Phe-Ala-Glu-Met-Ala-Ala-Ser-Leu-
Lys-Arg-Pro-Asp-Ser-Gly-Ala-Thr

-5-

Hybridoma supernatants were screened by ELISA assay. Immunofluorescence was performed on cryostat sections, which had previously been digested with testicular hyaluronidase (3.3 mg/ml in phosphate buffer pH 6.0) for 0.5 hour at 37°C. Primary antibody incubations were for 1 hour at room temperature. Following washes (3x5 min), the secondary antibody was applied (fluorescein-goat anti-mouse IgG). Culture supernatants from non-antibody producing hybridomas were collected and utilized as negative control for primary antisera.

Ten hybridoma strains were selected by such screening for each of the Example 1 and Example 2 oligopeptides. A specimen of the culture of each of the ten strains was injected into a pristane-primed mouse, the ascites collected, and the monoclonal antibody purified from the ascites by precipitation in 40% ammonium sulfate, then chromatographed on a column containing DEAE Sephacel. The antibodies were finally subjected to electrophoresis on SDS-polyacrylamide gels for assessment of purity. All monoclonal antibodies were determined to be of the IgG class. They were of various subclasses and types as follows:

IgG₁/κ;
IgG_{2A}/κ;
IgG_{2B}/κ;

All of the ten purified monoclonal antibodies against each of Examples 1 and 2 were tested for immunoreactivity with human α1 (IX) collagen chains by the following procedure. Specimens of costal human cartilage were frozen in liquid nitrogen, were smashed to

-7-

from within the amino-terminal globular portion of the human $\alpha 1(\text{IX})$ chain as shown in Fig. 1 can also be synthesized by conventional solid state synthesis:

5 Glu-Tyr-Ser-Phe-Leu-Thr-Thr-Phe-
 Arg-Met-Thr-Gly

 Phe-Arg-Ile-Pro-Thr-Arg-Asn-Leu-Tyr-
 Pro-Ser-Gly

10 In each case the oligopeptide can be coupled to a carrier protein by adding a single cysteinyl moiety to the carboxyl terminus, then using a conventional cross-linking agent. These carrier-supported oligopeptides can then be used as antigens in a manner analogous to that of Examples 1 and 2 to produce monoclonal antibodies.

15 The monoclonal antibodies immunoreactive with human Type IX collagen and human $\alpha(\text{IX})$ collagen chains can be labeled by any conventional procedure with any suitable label and employed in a conventional assay procedure for detecting the presence or absence of human Type IX collagen, $\alpha 1(\text{IX})$ collagen chains or fragments
20 thereof in a specimen of biological fluid such as serum, synovial fluid or the like. The biological fluid to be assayed can be assayed by ELISA assays, radioimmunoassays or any other conventional immunoassays using the monoclonal antibodies.

1/2

FIG. 1A

Gly Ala Lys Gly Val Ala Gly Glu Lys Gly Ser Thr Gly Ala Pro
 Gly Lys Pro Gly Gln Met Gly Asn Ser Gly Lys Pro Gly Gln Gln
 Gly Pro Pro Gly Glu Val Gly Pro Arg Gly Pro Arg Gly Leu Pro
 Gly Ser Arg Gly Glu Leu Gly Pro Val Gly Ser Pro Gly Leu Pro
 Gly Lys Leu Gly Ser Leu Gly Ser Pro Gly Leu Pro Gly Leu Pro
 Gly Pro Pro Gly Leu Pro Gly Met Lys Gly Asp Arg Gly Val Val
 Gly Glu Pro Gly Pro Lys Gly Glu Gln Gly Ala Ser Gly Glu Glu
 Gly Glu Ala Gly Glu Arg Gly Glu Leu Gly Asp Ile Gly Leu Pro
 Gly Pro Lys Gly Ser Ala Gly Asn Pro Gly Glu Pro Gly Leu Arg
 Gly Pro Glu Gly Ser Arg Gly Leu Pro Gly Val Glu Gly Pro Arg
 Gly Pro Pro Gly Pro Arg Gly Val Gln Gly Glu Gln Gly Ala Thr
 Gly Leu Pro Gly Val Gln Gly Pro Pro Gly Arg Ala Pro Thr Asp
 Gln His Ile Lys Gln Val Cys Met Arg Val Ile Gln Glu His Phe
 Ala Glu Met Ala Ala Ser Leu Lys Arg Pro Asp Ser Gly Ala Thr
 Gly Leu Pro Gly Arg Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro
 Gly Glu Asn Gly Phe Pro Gly Gln Met * * * * *
 * * * * *
 Gly Asp Leu Gly Glu Lys Gly Glu Arg Gly Pro Pro Gly Arg Gly
 Pro Asn Gly Leu Pro Gly Ala Ile Gly Leu Pro Gly Asp Pro Gly
 Pro Ala Ser Tyr Gly Lys Asn Gly Arg Asp Gly Glu Arg Gly Pro
 Pro Gly Leu Ala Gly * * * * *
 * * * * *
 * * * * *

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US90/00283

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC
 IPC (5) : C12P 21/02; C12S 3/16
 U.S. CI : 530/327,356

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System

Classification Symbols

U.S.

530/327,356

Documentation Searched other than Minimum Documentation
 to the Extent that such Documents are Included in the Fields Searched *

Chemical Abstracts Services Online (File CA, 1967-1988; File Biosis
 Previews 1969-1988). Automated Patent System (File US PAT, 1975-1988).

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category ⁸	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Journal of Biological Chemistry, Volume 263, No. 32, Issued November 1988, P. Bruckner, "The Structure of Human Collagen Type IX and its Organization in Fetal and Infant Cartilage Fibrils", See Pages 16911-16912.	1

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

Date of Mailing of this International Search Report

13 April 1990

04 MAY 1990

International Searching Authority

Signature of Authorized Officer

ISA/US

Laurie A. Scheiner

CONTINUATION SHEET - FORM PCT/ISA/210

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

- I. Claim 1 is drawn to an Oligopeptide, Classified in Class 530, Subclass 327.
- II. Claim 2 is drawn to an Oligopeptide, Classified in Class 530, Subclass 326.
- III. Claims 3-5 are drawn to an Antibody and a Method of Use, Classified in Class 435, Subclass 7.

Group I is directed to an Oligopeptide Product while Group II is directed to a separate and distinct Oligopeptide; Group III is directed to a separate and distinct immunological Method. The Oligopeptides of Group I and II could be used in a materially different method than that of Group III such as affinity chromatography. The method of Group III uses a product different from that of Groups I and II. The search burden involved for each additional invention is undue, not only insofar as the additional patent search, but also insofar as considerable Literature searches covering the entire class range noted above.